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An exciting novel member of *Lentitheciaceae* in Italy from *Clematis vitalba*

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Abstract – Dothideomycetes with muriform ascospores, were previously placed in family *Pleosporaceae*, but they are not a monophyletic group, and can be placed across a range of orders and families. In this study an interesting saprobic ascomycete was isolated from *Clematis vitalba* which was collected in Italy. The species has unique characters and we introduced the taxon as a new genus and species within *Lentitheciaceae,* The conclusions are drawn from morphology and, LSU, SSU, EF1-α and RPB2 combined sequence analyses. Maximum parsimony (MP), maximum likelihood (ML) and Mr Bayes phylogenetic analysis all support this being a distinct genus within the *Lentitheciaceae*. It is distinguished from other genera of this family in having muriform ascospores whose central cells have longitudinal septa and light end cells, and ascomata with a thick peridium and a short neck. The new genus is compared with similar genera in the *Lentitheciaceae* and a comprehensive description, and micrographs are provided. The cultures were obtained via single ascospore isolation, and the asexual state was also established.

***Lentitheciaceae* / new genus / *Murilentithecium* / new species / Italy**

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INTRODUCTION

We are studying Dothideomycetes with muriform ascospores in order to establish a natural classification based on multigene phylogeny (Nelsen *et al*. 2009, Schoch *et al*. 2009b, 2011, Boonmee *et al*. 2011, 2012, Chomnunti *et al*. 2011, 2014, Liu *et al*. 2011, 2012, Zhang *et al*. 2011, 2012, Hyde *et al*. 2013, Ariyawansa *et al.* 2014). In the most recent arrangement of Dothideomycetes, there were 23 orders incorporating 110 families (Wijayawardene *et al*. 2014).

Pleosporales is the largest orders of Dothideomycetes (Kirk *et al*. 2008; Schoch *et al.* 2009; Hyde *et al*. 2013) and comprises two suborders, Pleosporineae and Massarineae (Hyde *et al*. 2013, Zhang *et al.* 2012). Pleosporineae, is a phylogenetically well-supported suborder of Pleosporales and is characterized by broad to narrowly oblong ascomata, downwardly growing pseudoparaphyses, with 1-multiseptate ascospores (Zhang *et al.* 2012, Hyde *et al*. 2013). The suborder includes nine families: *Cucurbitariaceae*, *Didymellaceae*, *Didymosphaeriaceae*, *Dothidotthiaceae*, *Halojulellaceae*, *Leptosphaeriaceae*, *Phaeosphaeriaceae*, *Pleosporaceae* and *Shiraiaceae* (Ariyawansa *et al*. 2013a, Liu *et al*. 2013, Hyde *et al*. 2013). Massarineae is characterized by immersed or superficial ascomata, cylindrical asci with a short pedicel and 1-multiseptate ascospores (Zhang *et al*. 2012). The order currently includes six families: *Bambusicolaceae*, *Lentitheciaceae*, *Massarinaceae*, *Montagnulaceae*, *Morosphaeriaceae* and *Trematosphaeriaceae* with 20 genera whose species are mostly saprobic in terrestrial or aquatic environments (Hyde *et al*. 2013, Zhang *et al*. 2012).

The aim of this paper is to introduce a new genus/species in *Lentitheciaceae* which was discovered as a saprobe on *Clematis vitalba* in Italy. Combined gene (LSU, SSU, EF1-α and RPB2) analyses using maximum-likelihood (ML), maximum-parsimony (MP) and MrBayes clearly showed this species groups in *Lentitheciaceae* with high statistical support.

MATERIALS AND METHODS

Sample collection, morphological studies and isolation

The specimens were collected from two different sites in Italy. Specimens were brought to the laboratory in Zip lock plastic bags and examined under a Motic SMZ 168 stereomicroscope. Micromorphological characters were examined under a Nikon ECLIPSE 80i compound microscope and images were captured using a Nikon ECLIPSE 80i compound microscope with a Canon EOS 550D digital camera. India ink was added to water mounts to show the presence of a gelatinous sheath around the ascospores.

Single ascospore isolation was carried out following the method described in Chomnunti *et al*. (2014). Germinating ascospores were transferred aseptically to malt extract agar (MEA) plates and grown at 16°C in the daylight. Colony colour and other characters were observed and measured after a week and again after three weeks. The specimens are deposited in the Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. Living cultures are also deposited at the Culture Collection at Mae Fah Luang University (MFLUCC) and Mycothèque de l'Université catholique de Louvain, Belgium (MUCL).

DNA extraction, PCR amplification, sequencing and sequence alignment

Total fungal DNA was extracted from fresh fungal mycelium grown on PDA media at 16°C for four weeks using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux, P.R. China) following the instructions of the manufacturer.

Phylogenetic analyses were conducted using partial sequences of four genes, the internal transcribed spacers (5.8S, ITS), small subunit rDNA (18S, SSU), large subunit (28S, LSU), translation elongation factor 1-alpha gene (TEF 1α) and second largest subunit of RNA polymerase II (RPB2). Nuclear ITS was amplified using the primers ITS5 and ITS4 (White *et al*. 1990), LSU was amplified using the primers LROR and LR5 (Vilgalys and Hester 1990), SSU was amplified using the primers NS1 and NS4 (White *et al*. 1990), TEF was amplified using primers EF1-983F and EF1-2218R (Rehner 2001) and RPB2 was amplified using the primers fRPB2-5F and fRPB2-7cR (Liu *et al*. 1999).

Polymerase chain reaction (PCR) was carried out using the following protocol: The final volume of the PCR reaction was 25 µl and contained 12.5 µl of 2 x Power Taq PCR MasterMix (a premix and ready to use solution, including 0.1 Units/µl Taq DNA Polymerase, 500 µm dNTP Mixture each (dATP, dCTP, dGTP, dTTP), 20 mM Tris-HCL pH8.3, 100 Mm KCl, 3 mM $MgCl_2$, stabilizer and enhancer), 1 µl of each primer (10 µm), 1 µl genomic DNA extract and 9.5 µl deionised water. The reaction was then allowed to run for 35 cycles. The annealing temperature was 52.5°C for ITS, LSU, TEF and 48°C for SSU and initially 95°C for 5 mins, denaturation at 95°C for 90 seconds, annealing for 90 seconds, elongation at 72°C for 1 mins, and final extension at 72°C for 10 mins. The PCR thermal cycle program for the partial RNA polymerase second largest subunit (*RPB2*) was followed as initially 95°C for 5 mins., followed by 40 cycle of denaturation at 95°C for 1 mins, annealing at 52°C for 2 mins., elongation at 72°C for 90 seconds, and final extension at 72°C for 10 mins. PCR amplification was confirmed on 1% agarose electrophoresis gels stained with ethidium bromide. The amplified PCR fragments were sent to a commercial sequencing provider (Beijing Bai Mai Hui Kang Biological Engineering Technology Co. Ltd, P.R. China). The nucleotide sequence data acquired were deposited in GenBank (Table 1).

The other sequences used in the analyses (Table 1) were obtained from GenBank. The multiple alignments were automatically done by MAFFT v. 7.036 (Katoh and Standley 2013), but manual adjustments for improvement were made by eye where necessary using BioEdit v. 7.2 (Hall, 1999) and ClustalX (Kohli and Bachhawat 2013).

Phylogenetic analysis

The closest taxa to our strain were determined with standard nucleotide blast searches against the nucleotide database in GenBank (http://www.ncbi.nlm.nih.gov/), and sequences of representative species were selected from Gruyter *et al.* (2009), Hirayama *et al.* (2010), Hu *et al.* (2010), Hyde *et al*. (2013), Suterong *et al.* (2009), Quaedvlieg *et al.* (2013), Schoch *et al.* (2009), Shearer *et al.* (2009), Tanaka *et al.* (2009), Verkley *et al.* (2014) and Zhang *et al.* (2009a).

Combined analysis of LSU, SSU, EF1-α and RPB2 closest relatives in *Bambusicolaceae*, *Massarinaceae*, *Montagnulaceae*, *Morosphaeriaceae* and *Trematosphaeriaceae* were used to confirm the phylogenetic placement in suborder Massarineae in Pleosporales. *Pleospora herbarum* P. Karst was selected as an outgroup (Figure 1).

Table 1. Taxa used in the phylogenetic analysis and their corresponding GenBank numbers. The newly generated sequences are indicated in bold

Taxon	*Culture Accession No*	*GenBank Accession No.*				*References*
		LSU	*SSU*	*RPB2*	*TEF1*	
Bambusicola bambusae D.Q. Dai & K.D. Hyde	MFLUCC 11-0614^{T}	JX442035	JX442039	–	–	Dai *et al.* 2012
Bambusicola irregulispora D.Q. Dai & K.D. Hyde	MFLUCC 11-0437^{T}	JX442036	JX442040	–	–	Dai *et al.* 2012
Bambusicola splendid D.Q. Dai & K.D. Hyde	MFLUCC 11-0439^{T}	JX442038	JX442042	–	–	Dai *et al.* 2012
Bimuria novae-zelandiae D. Hawksw., Chea & Sheridan	CBS 107.79	AY016356	AY016338	DQ470917	DQ471087	Lumbsch and Lindemuth 2001
Byssothecium circinans Fuckel	CBS 675.92	AY016357	AY016339	DQ767646	GU349061	Hu *et al.* 2009
Deniquelata barringtoniae Ariyawansa & K.D. Hyde	MFLUCC 11-0422^{T}	JX254655	JX254656	–	–	Ariyawansa *et al.* 2013b
Falciformispora lignatilis K.D. Hyde	BCC 21118	GU371827	GU371835	–	GU371820	Ahmed *et al.* 2013
Falciformispora lignatilis K.D. Hyde	BCC 21117	GU371826	GU371834	–	GU371819	Ahmed *et al.* 2013
Halomassarina thalassiae (Kohlm. & Volkm.-Kohlm.) Suetrong, Sakay., E.B.G. Jones, Kohlm., Volkm.-Kohlm. & C.L. Schoch	JK 5262D^{T}	GU301816	–	–	GU349011	Schoch *et al.* 2009
Helicascus nypae K.D. Hyde	BCC 36752^{T}	GU479789	GU479755	GU479827	GU479855	Suetrong *et al.* 2009
Helicascus nypae K.D. Hyde	BCC 36751^{T}	GU479788	GU479754	GU479826	GU479854	Suetrong *et al.* 2009
Kalmusia brevispora (Nagas. & Y. Otani) Y. Zhang ter, Kaz. Tanaka & C.L. Schoch	KT 2313	AB524601	AB524460	AB539100	AB539113	Tanaka *et al.* 2009
Kalmusia brevispora (Nagas. & Y. Otani) Y. Zhang ter, Kaz. Tanaka & C.L. Schoch	KT 1466	AB524600	AB524459	AB539099	AB539112	Tanaka *et al.* 2009
Kalmusia scabrispora (Teng) Kaz. Tanaka, Y. Harada & M.E. Barr	KT 2202	AB524594	AB524453	AB539094	AB539107	Tanaka *et al.* 2009
Karstenula rhodostoma (Alb. & Schwein.) Speg.	CBS 690.94	GU301821	GU296154	GU371788	GU349067	Schoch *et al.* 2009
Katumotoa bambusicola Kaz. Tanaka & Y. Harada	KT 1517a^{T}	AB524595	AB524454	AB539095	AB539108	Tanaka *et al.* 2009
Keissleriella cladophila (Niessl) Corbaz	CBS 104.55^{T}	GU301822	GU296155	GU371735	GU349043	Verkley *et al.* 2014
Keissleriella genistae (Fuckel) E. Müll.	CBS 113798^{T}	GU205222	GU205242	–	–	Hu *et al.* 2009
Keissleriella rara Kohlm., Volkm.-Kohlm. & O.E. Erikss.	CBS 118429^{T}	GU479791	GU479757	–	–	Suetrong *et al.* 2009
Lentithecium aquaticum Ying Zhang, J. Fourn. & K.D. Hyde	CBS 123099^{T}	GU301823	GU296156	GU371789	GU349068	Schoch *et al.* 2009
Lentithecium arundinaceum (Sowerby) K.D. Hyde, J. Fourn. & Ying Zhang	CBS 123131^{T}	GU456320	GU456298	–	GU456281	Zhang *et al.* 2009a

Table 1. Taxa used in the phylogenetic analysis and their corresponding GenBank numbers. The newly generated sequences are indicated in bold *(continued)*

Taxon	*Culture Accession No*	*GenBank Accession No.*				*References*
		LSU	*SSU*	*RPB2*	*TEF1*	
Lentithecium fluviatile (Aptroot & Van Ryck.) K.D. Hyde, J. Fourn. & Ying Zhang	CBS 123090	FJ795450	FJ795492	FJ795467	–	Zhang *et al.* 2009b
Lentithecium fluviatile (Aptroot & Van Ryck.) K.D. Hyde, J. Fourn. & Ying Zhang	CBS 122367^{T}	FJ795451	FJ795493	–	GU456290	Schoch *et al.* 2009
Lentithecium lineare (E. Müll. ex Dennis) K.D. Hyde, J. Fourn. & Ying Zhang	IFRD 2008	FJ795435	FJ795478	–	–	Zhang *et al.* 2009b
Massarina cisti S.K. Bose	CBS 266.62^{T}	FJ795447	FJ795490	FJ795464		Zhang *et al.* 2009b
Massarina eburnean (Tul. & C. Tul.) Sacc.	CBS 473.64	GU301840	GU296170	GU371732	GU349040	Schoch *et al.* 2009
Montagnula opulent (de Not.) Aptroot	CBS 168.34	DQ678086	AF164370	DQ677984	–	Schoch *et al.* 2006
Morosphaeria ramunculicola (K.D. Hyde) Suetrong, Sakay., E.B.G. Jones & C.L. Schoch	JK 5304B^{T}	GU479794	GU479760	GU479831	–	Suetrong *et al.* 2009
Murilentithecium clematidis Wanasinghe, Camporesi, E.B.G. Jones & K.D. Hyde	MFLUCC 14-0561	KM408758	KM408759	KM454446	KM454444	This study
Murilentithecium clematidis Wanasinghe, Camporesi, E.B.G. Jones & K.D. Hyde	MFLUCC 14-0562	KM408760	KM408761	KM454447	KM454445	This study
Neottiosporina paspali (G.F. Atk.) B. Sutton & Alcorn	CBS 331.37^{T}	EU754172	EU754073	GU371779	GU349079	Gruyter *et al.* 2009
Paraconiothyrium minitans (W.A. Campb.) Verkley	CBS 122788	EU754173	EU754074	GU371776	GU349083	Gruyter *et al.* 2009
Paraphaeosphaeria michotii (Westend.) O.E. Erikss.	CBS 591.73	GU456326	GU456305	GU456352	GU456267	Zhang *et al.* 2009a
Paraphaeosphaeria michotii (Westend.) O.E. Erikss.	CBS 652.86	GQ387581	GQ387520	GU456351	GU456266	Verkley *et al.* 2014
Pleospora herbarum P. Karst.	CBS 191.86	GU238160	GU238232	DQ247794	KC584731	Aveskamp *et al.* 2010, Schoch *et al.* 2006b
Setoseptoria phragmitis Quaedvlieg, Verkley & Crous	CBS 114966	KF251753	–	KF252255	KF253200	Quaedvlieg *et al.* 2013
Setoseptoria phragmitis Quaedvlieg, Verkley & Crous	CBS 114802^{T}	KF251752	–	KF252254	KF253199	Quaedvlieg *et al.* 2013
Tingoldiago graminicola K. Hiray. & Kaz. Tanaka	KH 68^{T}	AB521743	AB521726	–	–	Hirayama *et al.* 2010
Trematosphaeria pertusa Fuckel	CBS 122368^{T}	FJ201990	FJ201991	FJ795476	GU456276	Ahmed *et al.* 2013

Abbreviations: **BCC**: Belgian Coordinated Collections of Microorganisms: **CBS**: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **CPC**: Collection of Pedro Crous housed at CBS; **MFLUCC**: Mae Fah Luang University CultureCollection, Chiang Rai, Thailand; **IFRD**: IFRDCC: Culture Collection, International Fungal Research & Development Centre, Chinese Academy of Forestry, Kunming, China; **T**: ex-type/ex-epitype isolates: **JK**: J. Kohlmeyer; **KH**: Kazuyuki Hirayama; **KT**: Kazuaki Tanaka.

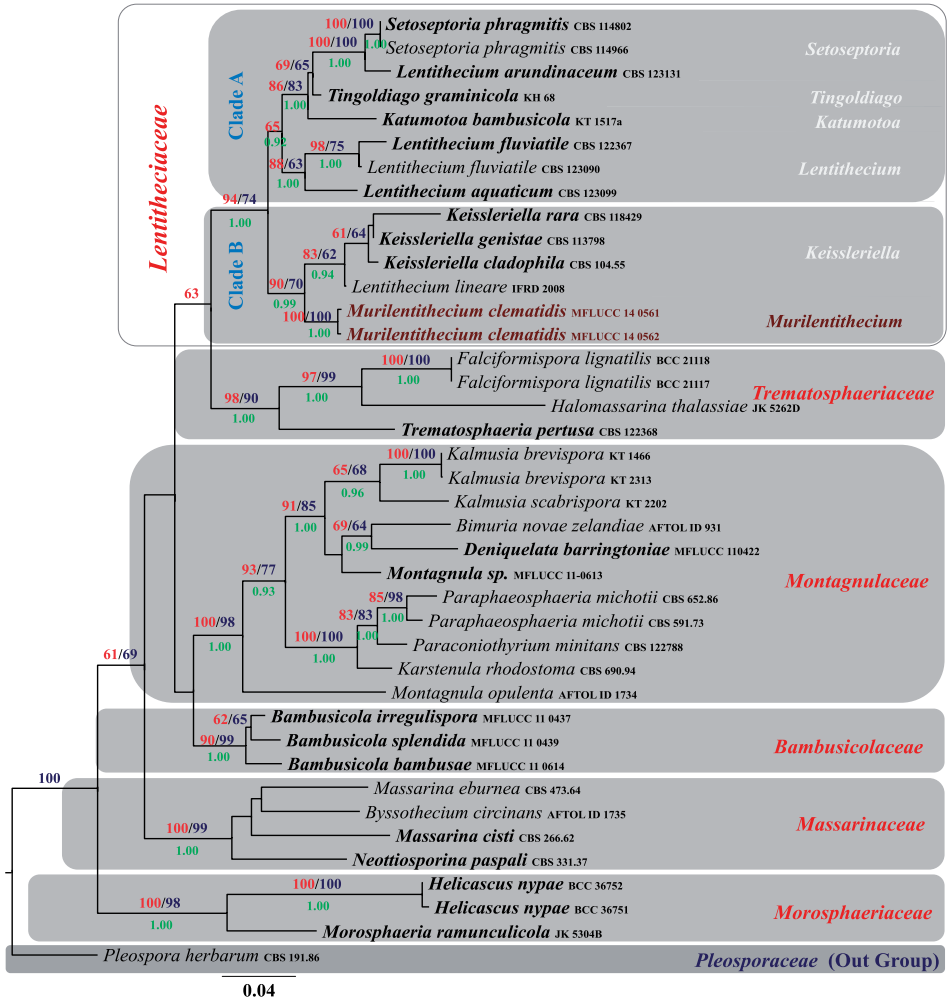

Fig. 1. RAxML tree based on a combined dataset of LSU, SSU, TEF and RBP2 partial sequences. Bootstrap support values for maximum parsimony (MP, blue) and maximum likelihood (ML, red) higher than 60% are defined as above the nodes. Bayesian posterior probabilities (BYPP, green) greater than 0.90 are provided below the nodes. The tree is rooted to *Pleospora herbarum* (CBS 191.86). All ex-type strains are in bold.

Parsimony analysis was carried with the heuristic search option in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002), with the following parameter settings: characters unordered with equal weight, random taxon addition, branch swapping with tree bisection-reconnection (TBR) algorithm, branches collapsing if the maximum branch length was zero, maxtrees set at 1000. Alignment gaps were treated as missing characters in the analysis of the combine data set, where they occurred in relatively conserved regions. Parsimony bootstrap analyses were performed using the full heuristic search

option, random stepwise addition, and 1000 replicates, with maxtrees set at 1000. Descriptive tree statistics for parsimony (Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI] were calculated for trees generated under different optimality criteria. The Kishino-Hasegawa tests (Kishino and Hasegawa 1989) were performed to determine whether the trees inferred under different optimality criteria were meaningfully different.

MODELTEST v. 3.7 (Posada and Crandall 1998) following Akaike Information Criterion was used to determine the best-fit model of evolution for each data set for Bayesian and Maximum Likelihood analyses.

Maximum-likelihood (ML) analysis was performed in RAxML (Stamatakis 2008) implemented in raxmlGUI v.0.9b2 (Silvestro and Michalak 2010), employing mixed models of evolution settings of the program and Bootstrap support obtained by running 1000 pseudo replicates.

A Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck and Ronqvist 2001) to valuate Posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). Two parallel runs were conducted, using the default settings, but with the following adjustments: Six simultaneous Markov chains were run and trees were sampled every 100th generation and the run was automatically stopped as soon as the average standard deviation of split frequencies reached below 0.01 (resulting 10000 total trees). The first 2000 trees were represented the burn-in phase of the analyses and discarded. The remaining 8000 trees were used for calculating PP in the majority rule consensus tree (Cai *et al*. 2006, 2008; Ariyawansa *et al*. 2013a). Maximum trees were visualized with Tree View (Page 1996).

RESULTS AND DISCUSSION

Phylogenetic analysis

The combined LSU, SSU, EF1-α and RPB2 data set comprised 40 sequences including our new strain of *Murilentithecium clematidis* with *Pleospora herbarum* (CBS 191.86) as the outgroup taxon. This analysis comprised 3675 characters, of which 2498 were constant, 957 parsimony informative and 220 parsimony-uninformative. Six equally parsimonious trees were generated and the first is selected (Figure 1). Bootstrap support (BS) values of ML and MP (equal to or above 60% based on 1000 replicates) are shown on the upper branches respectively with red and blue. Values of the Bayesian posterior probabilities (PP) from MCMC analyses are shown in green colour. The Kishino-Hasegawa test shows length = 3659 steps with CI = 0.477, RI = 0.636, RC = 0.303 and HI = 0.523.

Our strains of *Murilentithecium clematidis* (MFLUCC 14-0561 and 14-0562) grouped in *Lentitheciaceae*, but separated from the remaining genera of the family in a clade with relatively high bootstrap support (94%, Figure 1).

TAXONOMY

Lentitheciaceae Yin. Zhang *et al*., in Zhang *et al*., Stud. Mycol. 64: 93 (2009) **emended**

Saprobic on stems and twigs of herbaceous and woody plants in terrestrial or aquatic habitats. *Sexual state*: *Ascomata* scattered to gregarious,

immersed to superficial, globose to lenticular, dark brown to black, glabrous or with brown hyphae. *Ascomatal opening* short-papillate or undeveloped, central with or without brown short setae. *Peridium* composed of hyaline to brown, polygonal to angular, thin-walled cells. *Hamathecium* of cellular, septate and branched pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to broadly clavate, with a short pedicel, and a shallow ocular chamber at rounded apex, basal to somewhat lateral. *Ascospores* bi-seriate to tri-seriate, sometimes fasciculate, narrowly fusiform to broadly cylindrical, filiform in some species, straight or slightly curved, mostly 1-3-septate (murifom in some species), hyaline, smooth-walled, surrounded by an entire mucilaginous sheath or elongated appendage-like sheath. *Asexual morphs* stagonospora-like or dendrophoma-like. *Conidiomata* pycnidial, globose, ostiolate. *Conidiogenous cells* blastic or phialidic. *Conidia* cylindrical to oblong, hyaline to pigmented, one-celled to muriform.

Type species: ***Lentithecium fluviatile*** (Aptroot & Van Ryck.) K.D. Hyde *et al.*, in Zhang *et al.*, Fungal Divers. 38: 234 (2009), MycoBank: MB 512802

≡ *Massarina fluviatilis* Aptroot & Van Ryck., in Van Ryckegem and Aptroot, Nova Hedwigia 73: 162 (2001)

Murilentithecium Wanasinghe, Camporesi, E.B.G. Jones & K.D. Hyde, *gen. nov.*

Facesoffungi Number: FoF00293

Index Fungorum Number: IF550728

Etymology: The generic epithet is from the combination of two words *Muri* and *Lentithecium* meaning muriform ascospores in *Lentitheciaceae*

Type: *Murilentithecium clematidis* Wanasinghe, E. Camporesi, E.B.G. Jones & K.D. Hyde

A genus of *Lentitheciaceae*. *Saprobic* on dead herbaceous branches. *Sexual state*: *Ascomata* immersed, slightly erumpent, solitary, scattered, broadly oblong with a flattened base, dark brown to black, coriaceous, ostiolate. *Ostiole* papillate, blackish-brown, smooth, with ostiolar canal filled with pigmented cells. *Peridium* thick, comprising 8-10 layers, thick at the sides and thinner at the base, outer layer heavily pigmented, thick-walled, comprising blackish to dark brown cells of *textura angularis*, inner layer composed of hyaline, thin-walled cells of *textura angularis*. *Hamathecium* comprising numerous, filamentous, branched septate, pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, pedicellate, thick-walled at the apex, with a minute ocular chamber. *Ascospores* overlapping 1-2-seriate, muriform, mostly ellipsoidal, upper part wider than the lower part, 5-9 transversely septate, with 4-6 vertical septa, deeply constricted at the middle septum, initially hyaline, becoming yellowish-brown at maturity, smooth-walled, ends lighter, conical and narrowly rounded, surrounded by a thick, hyaline, mucilaginous sheath. *Asexual state*: *Conidiomata* pycnidial, solitary, dark brown, immersed, unilocular, with a papillate ostiole. Pycnidial wall multi-layered, with 3-4 outer layers of brown-walled cells of *textura angularis*, with inner most layer thin, hyaline. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* blastic, phialidic, hyaline, smooth, formed from the inner most layer of pycnidium wall. *Conidia* oblong, mostly straight, infrequently slightly curved, muriform, with 3-5 transverse septa, with 2-5 longitudinal septa, constricted at the septa, initially hyaline, pale brown to brown at maturity, narrowly rounded at both ends, smooth-walled.

Murilentithecium clematidis Wanasinghe, Camporesi, E.B.G. Jones & K.D. Hyde, *sp. nov.* **Figs 2-3**

Facesoffungi Number: FoF00294
Index Fungorum Number: IF550729
Etymology: Named after the host genus from which it was collected, *Clematis*

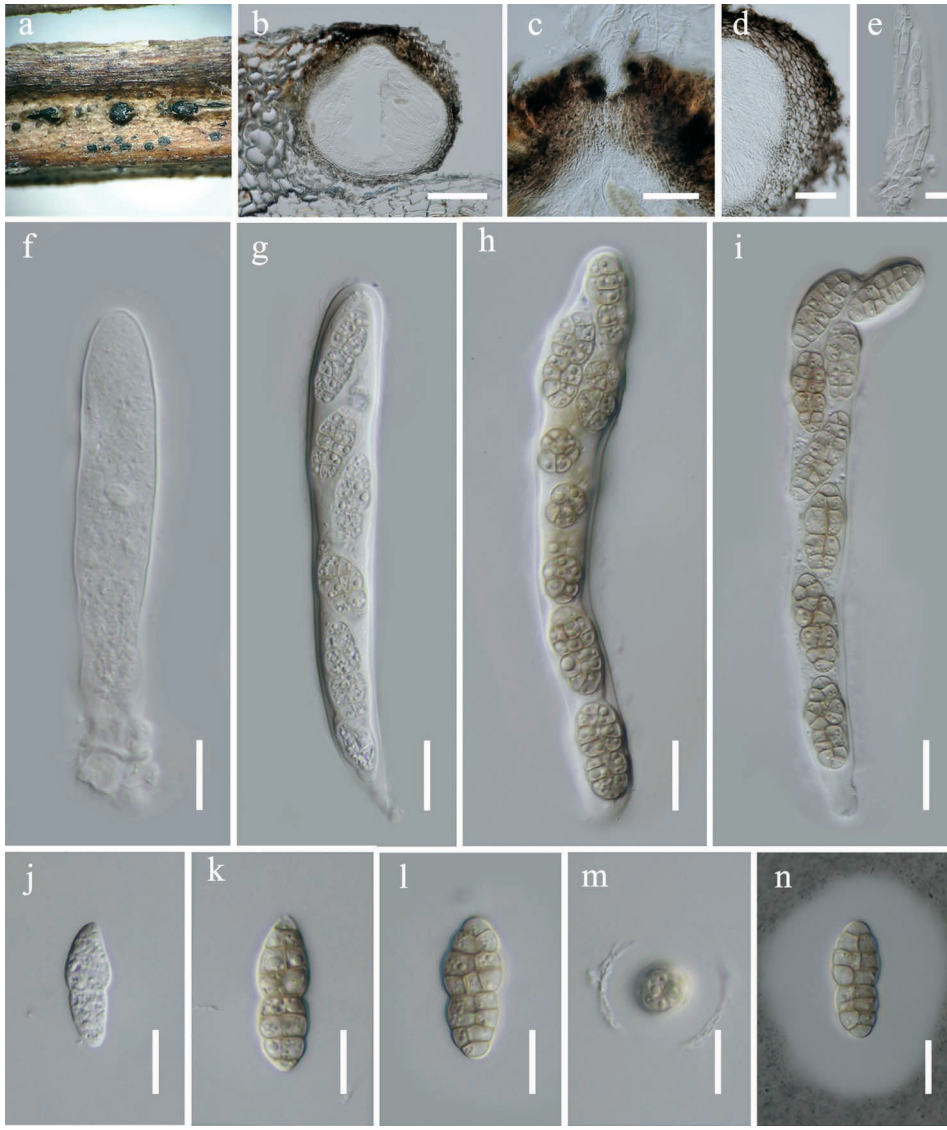

Fig. 2. *Murilentithecium clematidis* (holotype). **a.** Ascomata on host substrate. **b.** Section of ascoma. **c.** Close up of ostiole. **d.** Peridium. **e.** Pseudoparaphyses. **f-i.** Asci. **j-l.** Ascospores. **m.** An ascospore with the sheath viewed from apex. **n.** Ascospores stained with Indian ink. Note the lighter coloured end cells. Scale bars: b = 100 µm, c,d = 50 µm, e,f = 10 µm, g-i = 20 µm, j-n = 10 µm.

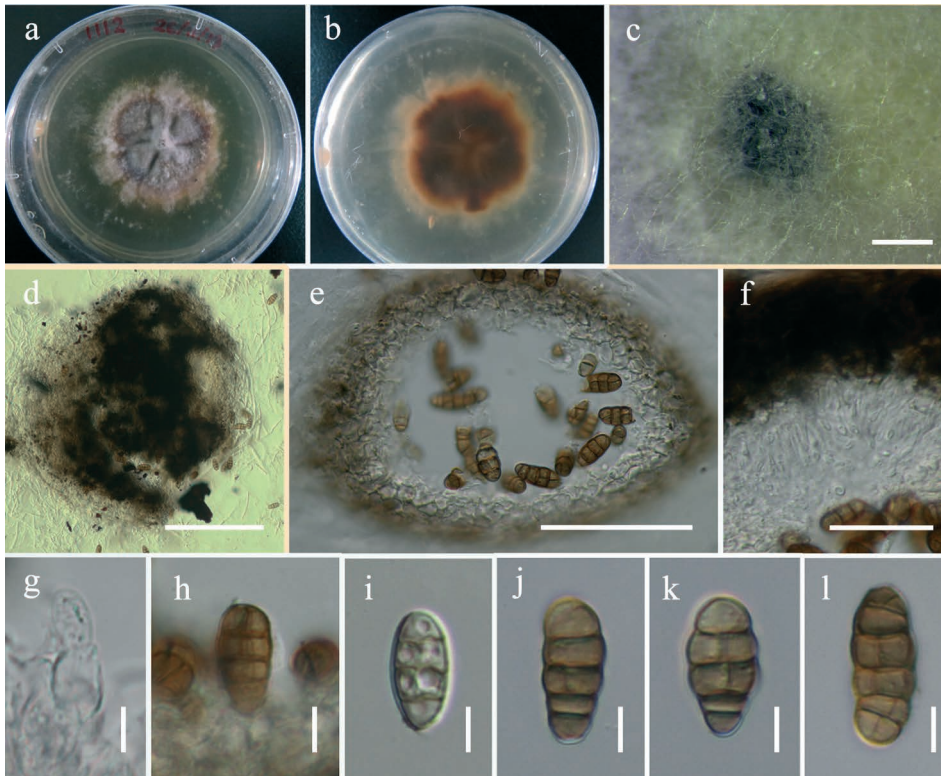

Fig. 3. ***Murilentithecium clematidis*** (holotype) **Asexual state**: **a, b.** Colonies on PDA (b from below). **c.** Immersed conidiomata. **d.** Squashed conidiomata. **e.** Longitudinal sections of conidiomata. **f.** Papillate ostiole. **g.** Immature and mature conidia attached to conidiogenous cell. **h-l.** Conidia. Scale bars: c = 500 µm, d = 100 µm, e = 50 µm, f = 20 µm, g-l = 5 µm.

Holotype: MFLU 14-0334

Saprobic on dead herbaceous branches. *Sexual state*: *Ascomata* 300-350 µm high 350-550 µm diam. ($\bar{x}$ = 321.5 × 461.8 µm, n = 10), slightly erumpent, solitary, scattered, hard to remove from the host substrate, with a flattened base, dark brown to black, coriaceous, ostiolate. *Ostiole* 50-80 µm high 45-60 µm diam. ($\bar{x}$ = 66.45 × 52.1 µm, n = 10) papillate, blackish brown, smooth, with ostiolar canal filled with brown cells. *Peridium* 40-50 µm wide at the base, 50-70 µm wide in sides, thick, comprising 8-10 layers, outer layer heavily pigmented, thick-walled, comprising blackish to dark brown cells of *textura angularis*, inner layer composed of hyaline, thin-walled cells of *textura angularis*. *Hamathecium* comprising numerous, 2.6 µm (n = 30) wide, filamentous, branched septate, pseudoparaphyses. *Asci* (90-130) × (10-20) µm ($\bar{x}$ = 15.1 × 114 µm, n = 40), 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, pedicellate, thick-walled at the apex, with minute ocular chamber. *Ascospores* (18-30) × (8-11) µm ($\bar{x}$ = 24.35 × 9.5 µm, n = 50), overlapping 1-2-seriate, muriform, mostly ellipsoidal, upper part wider than the lower part, 5-9 transversely septate, with 4-6 vertical septa, deeply constricted at the middle septum, initially hyaline, becoming yellowish-brown at maturity, ends lighter, conical and narrowly rounded at the ends, surrounded by a thick, hyaline,

mucilaginous sheath. *Asexual state*: *Conidiomata* 0.5-1.5 mm diam. pycnidial, solitary, dark brown, immersed, unilocular, with a papillate ostiole. Pycnidial wall (30-45 µm) multi-layered, with 3-4 outer layers of brown-walled cells of *textura angularis*, with inner most layer thin, hyaline. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* blastic, phialidic, hyaline, smooth, formed from the inner most layer of pycnidium wall. *Conidia* (13-18) × (5-8) µm ($\bar{x}$ = 15.6 × 6.5 µm, n = 50) oblong, mostly straight, infrequently slightly curved, muriform, with 3-5 transverse septa, with 2-5 longitudinal septa, constricted at the septa, initially hyaline, pale brown to brown at maturity, narrowly rounded at both ends, smooth-walled.

Colonies on PDA: slow growing, reaching 2 cm diam. after 3 weeks at 16°C, later with dense mycelium, circular, rough margin white at first, pinkish ash after 6 weeks (Fig. 3A-C) flat on the surface, without aerial mycelium. Hyphae septate branched, hyaline, thin. Sporulation after 8 weeks.

Known distribution: On dead branches of *Clematis vitalba* L. (*Ranunculaceae*), in Italy.

Material examined: ITALY, Arezzo Province: Badia Tega, Ortignano Raggiolo, dead and hanging branches of *Clematis vitalba*, 10 March 2013, E. Camporesi (MFLU 14-0334, **holotype**), — extype living culture = MFLUCC 14-0562 = MUCL STR/14-031B; Forlì-Cesena Province: Corniolo, Santa Sofia, dead and hanging branches of *Clematis vitalba*, 23 February 2013, E. Camporesi (MFLU 14-0335, **paratype**), — extype living culture = MFLUCC 14-0561 = STR/14-031A.

Gene sequence data: MFLUCC 14-0562: ITS (KM408757), LSU (KM408760), SSU (KM408761), RPB2 (KM454447) and TEF1α (KM454445); MFLUCC 14-0561: ITS (KM408756), LSU (KM408758), SSU (KM408759) RPB2 (KM454446) and TEF1α (KM454444).

DISCUSSION

Morphology

Generally lentitheceous taxa have narrow peridia, fusiform to broadly cylindrical hyaline ascospores with transverse septa (1-3-septate) containing refractive globules (Hyde *et al*. 2013; Zhang *et al*. 2012). *Murilentithecium* is characterized by having ascospores whose central cells have longitudinal septa, with light end cells, becoming yellowish-brown at maturity, a thick peridium and a short neck. Commonly the asexual state has cylindrical, hyaline conidia for lentitheceous genera (Hyde *et al*. 2013; Zhang *et al*. 2012). In our study we have observed brown, muriform conidia from MFLUCC 14-0561 and 14-0562 cultures. Consequently our new species/genus is morphologically distinct from other genera of *Lentitheciaceae.* Thus we introduce a new genus *Murilentithecium* to accommodate this fungus.

Phylogeny

In our combined gene analyses of Massarineae (Fig. 1), taxa from the family *Lentitheciaceae* formed a distinct clade with high bootstrap (94% and 74 in ML and MP analyses, respectively) and a high PP values (1.00 in Bayesian

analysis). *Setoseptoria* Quaedvlieg *et al*., *Tingoldiago* K. Hiray. & Kaz. Tanaka, *Katumotoa* Kaz. Tanaka & Y. Harada, *Lentithecium* K.D. Hyde *et al.* and *Keissleriella* Höhn. are grouped in *Lentitheciaceae* and the type species of the family is included in the analyses; thus we confirm their familial placement in *Lentitheciaceae.*

Our collection of *Murilentithecium clematidis*, is grouped in clade b (Fig. 1) of *Lentitheciaceae* with *Lentithecium lineare, Keissleriella genistae*, *K. rara* and *K. cladophila.* These species have distinct morphologies (Zhang *et al*. 2009b; 2012) and are separated from *Murilentithecium clematidis* with high support in the phylogenetic analysis (90% in ML analysis, 70 in MP analysis and 0.99 for Bayesian analysis).

Zhang *et al.* (2009b) introduced *Lentithecium lineare* (≡ *Keissleriella linearis* E. Müll. & Dennis) as a new combination in *Lentithecium* as it groups with *Lentithecium s. str.* However, they had not included any *Keissleriella* strains in their analyses (Zhang *et al.* 2009b). In our analyses (Fig. 1), *Lentithecium lineare* groups in *Keissleriella s. str*.

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REFERENCES

AHMED S.A., VAN DE SANDE W.W.J., STEVENS D.A., FAHAL A.H., DE HOOG G.S., 2014 — Taxonomic revision of agents of black-grain eumycetoma in the order Pleosporales. *Persoonia* (accepted).

ARIYAWANSA H., MAHARACHCHIKUMBURA S.S.N., KARUNARATHNE S.C., CHUKEATIROTE E., BAHKALI A.H., KANG J.K., BHAT D.J., HYDE K.D., 2013b — *Deniquelata barringtoniae gen. et sp. nov.*, associated with leaf spots of *Barringtonia asiatica*. *Phytotaxa* 105: 11-20.

ARIYAWANSA H.A., JONES E.B.G., SUETRONG S., ALIAS S.A., KANG J.C., HYDE K.D., 2013a — *Halojulellaceae* a new family of the order Pleosporales. *Phytotaxa* 130: 14-24.

ARIYAWANSA H.A., TANAKA K., THAMBUGALA K.M., PHOOKAMSAK R., TIAN Q., CAMPORESI E., HONGSANAN S., MONKAI J., WANASINGHE D.N., MAPOOK A., CHUKEATIROTE E., KANG J.C., XU J.C., MCKENZIE E.H.C., JONES E.B.G., HYDE K.D., 2014 — A molecular phylogenetic reappraisal of the *Didymosphaeriaceae* (= *Montagnulaceae*) *Fungal Diversity* (accepted).

AVESKAMP M.M., DE GRUYTER J., WOUDENBERG J.H., VERKLEY G.J., CROUS, P.W., 2010 — Highlights of the *Didymellaceae*: A polyphasic approach to characterise *Phoma* and related pleosporalean genera. *Studies in Mycology*. 65: 1-60.

BARR M.E., 1987 — New taxa and combinations in the *Loculoascomycetes*. *Mycotaxon* 29: 501-505.

BOONMEE S., KOKO T.W., CHUKEATIROTE E., HYDE K.D., CHEN H., CAI L., MCKENZIE E.H.C., JONES E.B.G., KODSUEB R., HASSAN B.A., 2012 — Two new *Kirschsteiniothelia* species with *Dendryphiopsis* anamorphs cluster in *Kirschsteiniotheliaceae* fam. nov. *Mycologia* 104: 698-714.

BOONMEE S., ZHANG Y., CHOMNUNTI P., CHUKEATIROTE E., TSUI C.K.M., BAHKALI A.H., HYDE K.D., 2011 — Revision of lignicolous Tubeufiaceae based on morphological reexamination and phylogenetic analysis. *Fungal Diversity* 51: 63-102.

CAI L., GUO X.Y., HYDE K.D., 2008 — Morphological and molecular characterization of a new anamorphic genus *Cheirosporium*, from freshwater in China. *Persoonia* 20: 53-58.

CAI L., JEEWON R., HYDE, K.D., 2006 — Phylogenetic investigations of Sordariaceae based on multiple gene sequences and morphology. *Mycological Research* 110: 137-150.

CHOMNUNTI P., HONGSANAN S., HUDSON B.A., TIAN Q., PERŠOH D., DHAMI M.K., ALIAS A.S., XU J., LIU X., STADLER M., HYDE K.D., 2014 — The Sooty Moulds. *Fungal Diversity* 66: 1-36.

CHOMNUNTI P., SCHOCH C.L., AGUIRRE-HUDSON B., KO-KO T.W., HONGSANAN S., JONES E.B.G., KODSUB R., CHUKEATIROTE E., BAHKALI A.H., HYDE K.D., 2011 — Capnodiaceae. *Fungal Diversity* 51: 103-134.

DAI D.Q., BHAT D.J., LIU J.K., ZHAO R.L. HYDE K.D., 2012 — *Bambusicola*, a new genus from bamboo with asexual and sexual morphs. *Cryptogamie Mycologie* 33: 363-379.

DE GRUYTER J.D., AVESKAMP M.M., WOUDENBERG J.H.C., VERKLEY G.J.M., GROENEWALD J.Z., CROUS P.W., 2009 — Molecular phylogeny of *Phoma* and allied anamorph genera: towards a reclassification of the *Phoma* complex. *Mycological Research* 113: 508-519.

HALL T.A., 1999 — BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: *Nucleic Acids Symposium Series* 41: 95-98

HIRAYAMA K., TANAKA K., RAJA H.A., MILLER A.N., SHEARER C.A., 2010 — A molecular phylogenetic assessment of *Massarina ingoldiana sensu lato*. *Mycologia* 102: 729-746.

HU H.L., FOURNIER J., JEEWON R., HYDE K.D., 2009 — Relationships among *Astrosphaeriella*, *Caryospora* and *Trematosphaeria*. PhD thesis, The University of Hong Kong, Hong Kong.

HUELSENBECK J.P. & RONQUIST F. 2001 — MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.

HYDE K.D., JONES E.B.G., LIU J.K, ARIYAWANSA H., BOEHM E., BOONMEE S., BRAUN U., CHOMNUNTI P., CROUS P.W., DAI D.Q., DIEDERICH P., DISSANAYAKE A., DOILOM M., DOVERI F., HONGSANAN S., JAYAWARDENA R., LAWREY J.D., LI Y.M., LIU Y.X., LÜCKING R., MONKAI J., MUGGIA L., NELSEN M.P., PANG K.L., PHOOKAMSAK R., SENANAYAKE I.C., SHEARER C.A., SUETRONG S., TANAKA K., THAMBUGALA K.M., WIJAYAWARDENE N.N., WIKEE S., WU H.X., ZHANG Y., AGUIRRE-HUDSON B., ALIAS S.A., APTROOT A., BAHKALI A., BEZERRA J.L., BHAT D.J., CAMPORESI E., CHUKEATIROTE E., GUEIDAN C., HAWKSWORTH D.L., HIRAYAMA K., HOOG S.D., KANG J.C., KNUDSEN K., LI W.J., LI X.H., LIU Z.Y., MAPOOK A., MCKENZIE E.H.C., MILLER A.N., MORTIMER P.E., PHILLIPS A.J.L., RAJA H.A, SCHEUER C., SCHUMM F., TAYLOR J.E., TIAN Q., TIBPROMMA S., WANASINGHE D.N., WANG Y., XU J.C., YACHAROEN S., YAN J.Y., ZHANG M., 2013 — Families of Dothideomycetes. *Fungal Diversity* 63: 1-313.

KATOH K. & STANDLEY K., 2013 — MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution* http://mbe.oxfordjournals.org/ by guest on June 22, 2013.

KIRK P.M., CANNON P.F., MINTER D.W., STALPERS J.A., 2008 — *Ainsworth & Bisby's Dictionary of the Fungi, 10th edn*. CABI, Wallingford, UK, pp. 485.

KISHINO H. & HASEGAWA M., 1989 — Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in hominoide. *Journal of Molecular Evolution* 29: 170-179.

KOHLI D.K. & BACHHAWAT A.K., 2003 — CLOURE: Clustal Output Reformatter, a program for reformatting ClustalX/ClustalW outputs for SNP analysis and molecular systematics. *Nucleic Acids Research* 31: 3501-3502.

KOHLMEYER J., VOLKMANN-KOHLMEYER B., ERIKSSON O.E., 1995 — Fungi on *Juncus roemerianus*. New marine and terrestrial ascomycetes. *Mycological Research* 100: 393-401.

LIU J.K., PHOOKAMSAK R., DOILOM M., WIKI S., MEI L.Y., ARIYAWANSA H.A., BOONMEE S., CHOMNUNTI P., DAI D.Q., BHAT D.J., ROMERO A.I., XHUANG W.Y., MONKAI J., JONES E.B.G,. CHUKEATIROTE E., KO-KO T.W., ZHOA Y.C., WANG Y., HYDE K.D., 2012 — Towards a natural classification of Botryosphaeriales. *Fungal Diversity* 57: 149-210.

LIU J.K., PHOOKAMSAK R., JONES E.B.G., ZHANG Y., KO-KO T.W., HU H.L., BOONMEE S., DOILOM M., CHUKEATIROTE E., BAHKALI A.H., WANG Y., HYDE K.D., 2011 — *Astrosphaeriella* is polyphyletic, with species in *Fissuroma gen. nov*., and *Neoastrosphaeriella gen. nov*. *Fungal Diversity* 51: 135-154.

LIU Y. J., WHELEN S. & HALL B.D., 1999 — Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* 16: 1799-1808.

LIU Y.X., HYDE K.D., ARIYAWANSA H.A., LI W.J., ZHOU D.Q., YANG Y.L., CHEN Y.M., LIU Z.Y., 2013 — *Shiraiaceae*, new family of Pleosporales (Dothideomycetes, Ascomycota). *Phytotaxa*, 103: 51-60.

LUMBSCH H.T. & LINDEMUTH R., 2001 — Major lineages of Dothideomycetes (Ascomycota) inferred from SSU and LSU rDNA sequences. *Mycological Research* 105: 901-908.

LUTTRELL E.S., 1955 — The ascostromatic Ascomycetes. *Mycologia* 47: 511-532.

NAGASAWA E. & OTANI Y., 1977 — Some species of the bambusicolous ascomycetes from Japan I. *Reports of the Tottori Mycological Institute* 15: 38-42.

NELSEN M.P., LÜCKING R., MBATCHOU J.S., ANDREW C.J., SPIELMANN A.A., LUMBSCH H.T., 2011 — New insights into relationships of lichen-forming Dothideomycetes. *Fungal Diversity* 51: 155-162.

NELSEN M.P., LÜCKING R., GRUBE M., MBATCHOU J.S., MUGGIA L., RIVAS-PLATA E., LUMBSCH H.T., 2009 — Unravelling the phylogenetic relationships of lichenised fungi in Dothideomyceta. *Studies in Mycology* 64: 135-144.

PAGE R.D.M., 1996 — Tree View: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12: 357-358.

POSADA D. & CRANDALL K.A., 1998 — Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

QUAEDVLIEG W., VERKLEY G.J.M., SHIN H.-D., BARRETTO R.W., ALFENAS A.C., SWART W.J., GROENEWALD J.Z., CROUS P.W., 2013 — Sizing up *Septoria*. *Studies in Mycology*. 75: 307-390.

RANNALA B, & YANG Z., 1996 — Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43: 304-311

REHNER S., 2001 — *Primers for Elongation Factor 1-α (EF1-α)*. http://ocid.NACSE.ORG/research/deephyphae/EF1primer.pdf.

SCHOCH C.L., SUNG G.H., LÓPEZ-GIRÁLDEZ F., TOWNSEND J.P., MIADLIKOWSKA J., HOFSTETTER V., ROBBERTSE B., MATHENY P.B., KAUFF F.,WANG Z., GUEIDAN C., ANDRIE R.M., TRIPPE K., CIUFETTI L.M., WYNNS A., FRAKER E., HODKINSON B.P., BONITO G., GROENEWALD J.Z., ARZANLOU M., DE HOOG G.S., CROUS P.W., HEWITT D., PFISTER D.H., PETERSON K., GRYZENHOUT M., WINGFIELD M.J., APTROOT A., SUH S.O., BLACKWELL M., HILLIS D.M., GRIFFITH G.W., CASTLEBURY L.A., ROSSMAN A.Y., LUMBSCH H.T., LÜCKING R., BÜDEL B., RAUHUT A., DIEDERICH P., ERTZ D., GEISER D.M., HOSAKA K., INDERBITZIN P., KOHLMEYER J., VOLKMANN-KOHLMEYER B., MOSTERT L., O'DONNELL K., SIPMAN H., ROGERS J.D., SHOEMAKER R.A., SUGIYAMA J.,SUMMERBELL R.C., UNTEREINER W., JOHNSTON P.R., STENROOS S., ZUCCARO A., DYER P.S., CRITTENDEN P.D., COLE M.S., HANSEN K., TRAPPE J.M., YAHR R., LUTZONI F., SPATAFORA J.W., SCHOCH C.L. *et al.*, 2009b — The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. *Systematic Biology* 58: 224-239.

SCHOCH C.L., CROUS P.W., GROENEWALD J.Z. *et al.*, 2009 — A class-wide phylogenetic assessment of Dothideomycetes. *Studies in Mycology* 64: 1-15.

SCHOCH C.L., SHOEMAKER R.A., SEIFERT K.A., HAMBLETON S., SPATAFORA J.W., CROUS P.W., 2006 — A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia* 98: 1041-1052.

SCHOCH C.L., KOHLMEYER J., VOLKMANN-KOHLMEYER B., TSUI C.K., SPATAFORA J.W., 2006b — The halotolerant fungus *Glomerobolus gelineus* is a member of the Ostropales. *Mycological Research* 110: 257-263.

SHEARER C.A., RAJA H.A., MILLER A.N., NELSON P., TANAKA K., HIRAYAMA K., MARVANOVA L., HYDE K.D., ZHANG Y., 2009 — The molecular phylogeny of freshwater Dothideomycetes. *Studies in Mycology* 64: 145-153.

SILVESTRO D. & MICHALAK I., 2012 — raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* 12: 335-337.

STAMATAKIS A., 2006 — RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688-2690.

SUETRONG S., SCHOCH C.L., SPATAFORA J.W., KOHLMEYER J., VOLKMANN-KOHLMEYER B., SAKAYAROJ J., PHONGPAICHIT S., TANAKA K., HIRAYAMA K., JONES E.B.G., 2009 — Molecular systematics of the marine Dothideomycetes. *Studies in Mycology* 64: 155-173.

SWOFFORD D.L. 2002 — PAUP: phylogenetic analysis using parsimony, version 4.0 b10. Sinauer Associates, Sunderland.

TANAKA K. & HARADA Y., 2005c — Bambusicolous fungi in Japan (6): *Katumotoa*, a new genus of phaeosphaeriaceous ascomycetes. *Mycoscience* 46: 313-318.

TANAKA K., HIRAYAMA K., YONEZAWA H., HATAKEYAMA S., HARADA Y., SANO T., SHIROUZU T., HOSOYA T., 2009 — Molecular taxonomy of bambusicolous fungi: *Tetraplosphaeriaceae*, a new *pleosporalean* family with *Tetraploa*-like anamorphs. *Studies in Mycology* 64: 175-209.

VERKLEY G.J.M., DUKIK K., RENFURM R., GÖKER M., STIELOW J.B., 2014 — Novel genera and species of *Coniothyrium*-like fungi in *Montagnulaceae* (Ascomycota) *Persoonia* 32: 25-51.

VILGALYS R. & HESTER M., 1990 — Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238-4246.

WHITE T., BRUNS T., LEE S., TAYLOR J., 1990 — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: *A Guide to Methods and Applications* 18: 315-322.

WIJAYAWARDENE N.N., CROUS P.W., KIRK P.M., HAWKSWORTH D.L. *et al.*, 2014 — Naming and outline of Dothideomycetes – 2014. *Fungal Diversity* (accepted).

ZHANG Y., SCHOCH C.L., FOURNIER J., CROUS P.W., DE GRUYTER J., WOUDENBERG J.H.C., HIRAYAMA K., TANAKA K., POINTING S.B., SPATAFORA J.W., HYDE K.D., 2009a — Multi-locus phylogeny of the Pleosporales: a taxonomic, ecological and evolutionary re-evaluation. *Studies in Mycology* 64: 85-102.

ZHANG Y., WANG H.K., FOURNIER J., CROUS P.W., JEEWON R., POINTING S.B., HYDE K.D., 2009b — Towards a phylogenetic clarification of *Lophiostoma*, *Massarina* and morphologically similar genera in the Pleosporales. *Fungal Diversity* 38: 225-251.

ZHANG Y., CROUS P.W., SCHOCH C.L., HYDE K.D., 2012 — *Pleosporales*. *Fungal Diversity* 52: 1-225.

ZHANG Y., CROUS P.W., SCHOCH C.L., BAHKALI A.H., GUO L.D., HYDE K.D., 2011 — A molecular, morphological and ecological re-appraisal of *Venturiales*-a new order of Dothideomycetes. *Fungal Diversity* 51: 249-277.

ZHAXYBAYEVA O. & GOGARTEN J.P., 2002 — Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. *BMC Genomics* 3(1): 4.